

71 sequence having at least 95% homology to SEQ ID NO:1, with an amino acid sequence from 1<sup>st</sup> His to 21<sup>st</sup> Gly of SEQ ID NO:2, wherein said mutant  $\alpha$ -amylase possess increased heat resistance and maintains resistance to chelating agents when compared to SEQ ID NO:1.

5. (Three Times Amended) A mutant  $\alpha$ -amylase obtained by introducing a first mutation and a second mutation into SEQ ID NO:1 or an amino acid sequence having at least 95% homology to SEQ ID NO:1,

72 wherein said first mutation consists of a substitution or a deletion of at least one amino acid residue selected from the group consisting of the 11<sup>th</sup> Tyr, 16<sup>th</sup> Glu, 49<sup>th</sup> Asn, 84<sup>th</sup> Glu, 144<sup>th</sup> Ser, 167<sup>th</sup> Gln, 169<sup>th</sup> Tyr, 178<sup>th</sup> Ala, 188<sup>th</sup> Glu, 190<sup>th</sup> Asn, 205<sup>th</sup> His and 209<sup>th</sup> Gln, and

wherein said second mutation consists of a substitution of a sequence corresponding to the 11<sup>th</sup> to 100<sup>th</sup> amino acid residue from the amino terminus of the amino acid sequence set forth in SEQ ID NO:1, and

wherein said mutant  $\alpha$ -amylase possesses increased heat resistance and maintains resistance to chelating agents when compared to SEQ ID NO:1.

6. (Twice Amended) The mutant  $\alpha$ -amylase according to Claim 5, wherein said first mutation consists of:

~~the substitution of an amino acid residue selected from the group consisting of: the 11<sup>th</sup> Tyr of SEQ ID NO:1 with Phe, the 16<sup>th</sup> Glu of SEQ ID NO:1 with Pro, the 49<sup>th</sup> Asn of SEQ ID NO:1 with Ser, the 167 Gln of SEQ ID NO:1 with Glu, the 169<sup>th</sup> Tyr of SEQ ID NO:1 with Lys, the 190<sup>th</sup> Asn of SEQ ID NO:1 with Phe, the 205<sup>th</sup> His of SEQ ID NO:1 with Arg, and the 209<sup>th</sup> Gln of SEQ ID NO:1 with Val,~~

*73*  
and wherein said second mutation consists of:

substituting an amino terminal sequence from 1<sup>st</sup> Asp through 19<sup>th</sup> Gly of SEQ ID NO:1 with an amino acid sequence from 1<sup>st</sup> His to 21<sup>st</sup> Gly of SEQ ID NO:2.

*74*  
13. (Amended) A mutant  $\alpha$ -amylase obtained by making a substitution or deletion of at least one amino acid residue of specific positions in SEQ ID NO:1, or by making a substitution or deletion of at least one amino acid residue corresponding to the above-mentioned amino acid residue in a sequence having at least 95% homology to SEQ ID NO:1,

wherein said at least one amino acid residue is selected from the group consisting of:

the 11<sup>th</sup> Tyr, 16<sup>th</sup> Glu, 49<sup>th</sup> Asn, 84<sup>th</sup> Glu, 144<sup>th</sup> Ser, 167<sup>th</sup> Gln, 169<sup>th</sup> Tyr, 178<sup>th</sup> Ala, 188<sup>th</sup> Glu, 190<sup>th</sup> Asn, 205<sup>th</sup> His and 209<sup>th</sup> Gln, and

wherein said mutant  $\alpha$ -amylase:

- (i) decomposes  $\alpha$ -1,4-glycoside bonds of starch, amylose, amylopectin, and partially decomposed products thereof;
- (ii) produces glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose from amylose;
- (iii) does not act on pullulan;
- (iv) exhibits a residual activity of at least 70% in a pH range of 6.5 to 11 under treatment conditions of 40°C and 30 minutes;
- (v) acts in a temperature range of 20°C to 80°C;
- (vi) exhibits a residual activity of at least 80% when incubated at 40°C, or at least 60% when incubated at 45°C, for 30 minutes in 50 mM glycine-sodium hydroxide buffer at pH 10;
- (vii) has a molecular weight of 55,000  $\pm$  5,000 as measured by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis;

(viii) has an isoelectric point of about 4.2 as measured by isoelectric focusing;

(ix) has a residual activity of at least 90% when treated at pH 10 and 30°C for 30 minutes in a 0.1% solution of a surfactant selected from the group consisting of:

sodium linear alkylbenzenesulfonates, sodium alkylsulfates, sodium polyoxyethylene alkylsulfates, sodium α-olefinsulfonates, sodium salts of α-sulfonated fatty acid esters, sodium alkylsulfonates, SDS, soap, and Softanol;

44  
(x) is inhibited by 1 mM Mn<sup>2+</sup> by about 75%, or by 1 mM Sr<sup>2+</sup> or 1 mM Cd<sup>2+</sup> by about 30 to 40%, when treated at pH 10 and 30°C for 30 minutes; and

(xii) comprises an amino acid sequence which is at least 70% homologous to SEQ ID NO:1.

---

Please add the following claims:

75  
--15. The mutant α-amylase according to claim 12, wherein the 11<sup>th</sup> Tyr of SEQ ID NO:1 is substituted with Phe, the 16<sup>th</sup> Glu of SEQ ID NO:1 is substituted with Pro, the 49<sup>th</sup> Asn of SEQ ID NO:1 is substituted with Ser, the 167 Gln of SEQ ID NO:1 is substituted with Glu, the 169<sup>th</sup> Tyr of SEQ ID NO:1 is substituted with Lys, the 190<sup>th</sup> Asn of SEQ ID NO:1 is substituted with Phe,

the 205<sup>th</sup> His of SEQ ID NO:1 is substituted with Arg, and the 209<sup>th</sup> Gln of SEQ ID NO:1 is substituted with Val.--

25  
--16. The mutant  $\alpha$ -amylase according to claim 13, wherein said mutant  $\alpha$ -amylase comprises an amino acid sequence which is at least 95% homologous to SEQ ID NO:1.--

--17. The mutant  $\alpha$ -amylase according to claim 13 or 16, wherein the 11<sup>th</sup> Tyr of SEQ ID NO:1 is replaced with Phe.--

--18. The mutant  $\alpha$ -amylase according to claim 13 or 16, wherein the 16<sup>th</sup> Glu of SEQ ID NO:1 is replaced with Pro.--

--19. The mutant  $\alpha$ -amylase according to claim 13 or 16, wherein the 49<sup>th</sup> Asn of SEQ ID NO:1 is replaced with Ser.--

--20. The mutant  $\alpha$ -amylase according to claim 13 or 16, wherein the 167 Gln of SEQ ID NO:1 is replaced with Glu.-

--21. The mutant  $\alpha$ -amylase according to claim 13 or 16, wherein the 169<sup>th</sup> Tyr of SEQ ID NO:1 is replaced with Lys.--

--22. The mutant  $\alpha$ -amylase according to claim 13 or 16, wherein the 190<sup>th</sup> Asn of SEQ ID NO:1 is replaced with Phe.--

--23. The mutant  $\alpha$ -amylase according to claim 13 or 16, wherein the 205<sup>th</sup> His of SEQ ID NO:1 is replaced with Arg.--

--24. The mutant  $\alpha$ -amylase according to claim 13 or 16, wherein the 209<sup>th</sup> Gln of SEQ ID NO:1 is replaced with Val.--

--25. A mutant  $\alpha$ -amylase obtained by introducing a mutation into SEQ ID NO:1,

*75*       wherein said mutation consists of:

the substitution of an amino acid residue selected from the group consisting of: the 11<sup>th</sup> Tyr, 16<sup>th</sup> Glu, 49<sup>th</sup> Asn, 84<sup>th</sup> Glu, 144<sup>th</sup> Ser, 167<sup>th</sup> Gln, 169<sup>th</sup> Tyr, 178<sup>th</sup> Ala, 188<sup>th</sup> Glu, 190<sup>th</sup> Asn, 205<sup>th</sup> His and 209<sup>th</sup> Gln, with another amino acid.--

--26. The mutant  $\alpha$ -amylase according to claim 25, wherein the 11<sup>th</sup> Tyr of SEQ ID NO:1 is substituted with Phe, the 16<sup>th</sup> Glu of SEQ ID NO:1 is substituted with Pro, the 49<sup>th</sup> Asn of SEQ ID NO:1 is substituted with Ser, the 167 Gln of SEQ ID NO:1 is substituted with Glu, the 169<sup>th</sup> Tyr of SEQ ID NO:1 is substituted with Lys, the 190<sup>th</sup> Asn of SEQ ID NO:1 is substituted with Phe,

the 205<sup>th</sup> His of SEQ ID NO:1 is substituted with Arg, and the 209<sup>th</sup> Gln of SEQ ID NO:1 is substituted with Val.-

--27. A mutant  $\alpha$ -amylase obtained by introducing a mutation into SEQ ID NO:1,

*75* and wherein said mutation consists of:

substituting an amino terminal sequence from 1<sup>st</sup> Asp through 19<sup>th</sup> Gly of SEQ ID NO:1 with an amino acid sequence from 1<sup>st</sup> His to 21<sup>st</sup> Gly of SEQ ID NO:2.--

---

Attached hereto is a marked-up version showing the changes made to the application by this Reply.